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Increased survival and migration of engrafted mesenchymal bone marrow stem cells in 6-hydroxydopamine-lesioned rodents

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Abstract

Parkinson's disease is characterized by the loss of dopaminergic neurons in the substantia nigra. Attempted replacement of these neurons by stem cells has proved inconclusive. Bone marrow mesenchymal stem cells (MSC) are multipotent, differentiating into a variety of cells, including neuron-like cells. We used the 6-hydroxydopamine (6-OHDA) animal model of Parkinson's disease to assess migration and differentiation of transplanted MSC. We found in rodents that transplanted MSC survive better in the 6-OHDA-induced damaged hemisphere compared to the unlesioned side. Moreover, contralaterally engrafted MSC migrated through the corpus callosum to populate the striatum, thalamic nuclei and substantia nigra of the 6-OHDA-lesioned hemisphere. In conclusion, we demonstrate that 6-OHDA-induced damage increases the viability of transplanted MSC and attracts these cells from the opposite hemisphere.

Keywords: Bone marrow stem cells; Parkinson's disease; 6-Hydroxydopamine

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta and the consequent loss of projecting nerve fibers in the striatum [2]. Although the presence of intrinsic neural stem cells that have the potential to renew to new neurons in response to injury has been well demonstrated to occur in the subventricular zone and hippocampus [6], this intrinsic repair is insufficient to prevent disease expression. Therefore, replacing degenerated neurons by exogenous potent stem cells that can generate unlimited numbers of cells, presents a more promising technique for tissue repair and regeneration in PD [18]. Fetal brain tissue has been transplanted in patients with PD, but results have proven inconclusive, and despite some clinical improvement, the use of fetal tissue is limited by ethical and logistical issues [9,20]. Transplantation of induced dopaminergic neurons derived from mouse embryonic stem cells has been shown to improve the neurological symptoms of rats with a 6-hydroxydopamine (6-OHDA) induced Parkinson-like syndrome [12]. Dopaminergic cells derived from CNS precursors however had limited survival and were not able to improve apomorphine-induced rotation scores in 6-OHDA lesioned rats [13]. Bone marrow derived stem cells, provide an alternative to embryonal stem cells, in that they are easily harvested, isolated, and purified and their use is not limited by ethical issues. It is now well established that apart form hematopoietic stem cells, bone marrow is composed of mesenchymal stem cells (MSC), that have proved to be multipotent cells with the potential to differentiate into a variety of cells, such as osteoblasts, chondrocytes and adipocytes [19]. Moreover, recent studies have shown that in vitro, MSC might be induced to undergo differentiation to neuron-like cells [15]. One of the vital processes that implanted stem cells have to undergo in order to influence damaged tissue is migration and differentiation. Although it has already been reported that stem cells have the capacity to migrate towards damaged tissues in the CNS [1], this has not been established in the animal model of PD. The aim of this study was, therefore, to investigate whether transplanted bone marrow derived stem cells survive in 6-OHDA-injured tissue, and if they have the capacity to migrate to and engraft in the lesioned striatum, when transplanted in the opposite cerebral hemisphere.

In the first experiment, 6-OHDA (4 μg) was injected into the right substantia nigra of rats (Sprang dolly rats, 250 g, $n = 6$) anterior 4.8 mm, lateral 1.8 mm, dorsoventral 8.1 mm, with respect to the bregma and the dura [11]. Fourteen days later, the lesioned

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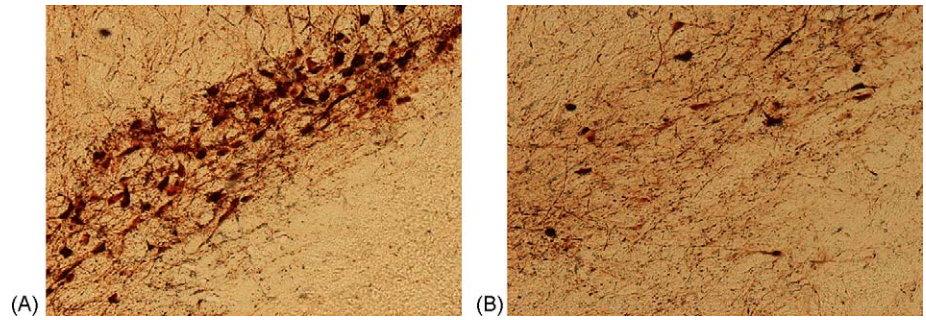

Fig. 1. Anti-tyrosine hydroxylase (TH) staining of rat substantia nigra following 6-OHDA lesion. (A) TH positive cells in an unlesioned control rat. (B) TH positive cells after 6-OHDA lesioning.

rats were tested for rotational behavior induced by an intraperitoneal injection of amphetamine (10 mg/kg), for a period of 1 h. This test confirms unilateral striatal or nigral damage. Only rats with proven rotational behavior (>5 rpm) were selected for brain transplantation of mouse bone marrow cells. Cells obtained from the femur and tibia of C57/Bl mice, were centrifuged and plated in polystyrene plastic culture flasks in a growth medium of DMEM supplemented with 15% fetal calf serum (FCS), 5% horse serum, 0.001%, beta-mercaptoethanol, 2 mM glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 12.5 units/ml nystatin (PSN). Cells were incubated for 48 h and nonadherent cells were removed. The cells, fibroblast-like in shape, were grown and expanded in flasks for several weeks, and demonstrated typical characteristics of mesenchymal cells including clonality, capability to induce to adipocytes and the absence of hematopoetical surface markers such as CD45.

The mouse bone marrow derived stem cells (10^5), were injected into the right and left rat striata ($n = 4$) 20 days after unilateral 6-OHDA lesion in the substantia nigra.

Forty-five days later, the rats were sacrificed. The 6-OHDA lesion in the substantia nigra was verified with anti-tyrosine hydroxylase staining (Fig. 1). Immunohistochemistry of rat tissue was performed with rat anti-mouse antigen antibodies (M6, 1:200, v/v, Developmental Studies Hybridoma Bank, DSHB) followed by rabbit anti-rat HRP. The number of M6-immunopositive cells demonstrated a significantly higher survival rate of mouse cells in the right hemisphere, the 6-OHDA injected side, compared to the left, unlesioned, hemisphere (Fig. 2). Thus, the unilateral 6-OHDA lesion in the nigra, followed by destruction of the dopaminergic terminals in the striatum, increased the survival of the engrafted cells.

To address the question whether the damaged striata might release factors that attract the transplanted cells to the lesion, we injected 6-OHDA to the right striatum of C57/Bl mice, while MSC and MSC that were induced to differentiate to neuron like cells, were transplanted into the left striatum. Two microliters of 6-OHDA was injected stereotactically into the right striatum using co-ordinates from the Stereotaxis Atlas [16] anterior

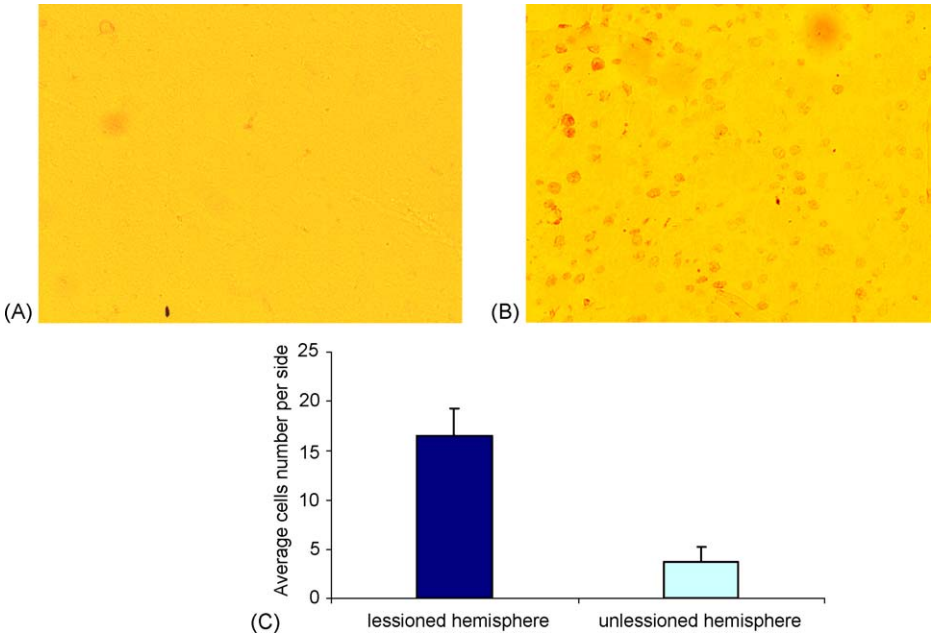

Fig. 2. Transplanted bone marrow mesenchymal cells in the rat striatum following a 6-OHDA lesion of the substantia nigra on the right side. (A) Immunostaining with mouse antigen (M6) of mouse bone marrow cells in the left striatum (unlesioned side). (B) Immunostaining with M6 of mouse bone marrow cells in the right striatum (lesioned side). (C) Quantification of transplanted cells visualized by anti-mouse antigens (M6). (Average of 75 slides received from four rats in each group.)

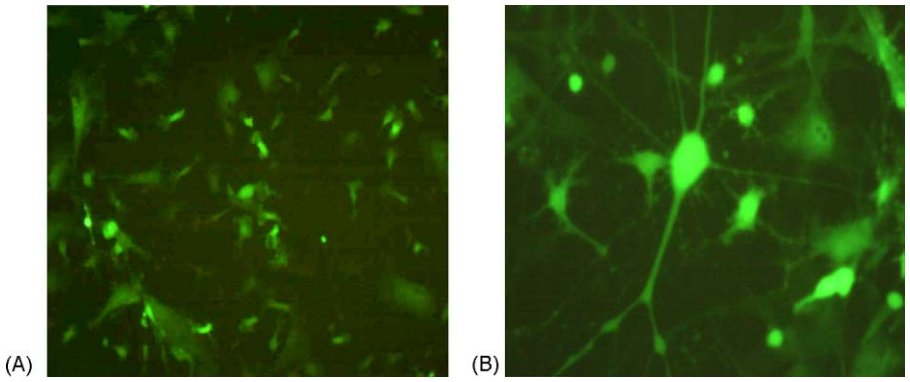

Fig. 3. Induction of differentiation of mouse bone marrow mesenchymal cells into neuron-like cells. (A) Mouse bone marrow mesenchymal cells after 3 weeks in culture. (B) Neuron-like cells after differentiation.

1.1 mm, lateral −2.3 mm, dorsoventral −4.2 mm, with respect to the bregma and the dura. Note that only mice with proven rotational behavior (>5 rpm) were selected for brain transplantation. Twenty days after lesioning, we transplanted MSC and neuronal-differentiated MSC (0.2×10^6) obtained from the femur and tibia of transgenic mice bearing the enhanced green fluorescent protein (Tg-GFP) [8]. Neural differentiation was then performed as we previously described [15], with DMEM supplemented with 10% FCS, 10 ng/ml basic fibroblast growth factor, 10 ng/ml epidermal growth factor and 10 ng/ml N2 solution for a further 24 h. The medium was then changed to one composed of DMEM supplemented with 200 μM butylated hydroxyanisole, 1 mM dibutyryl cyclic AMP, 0.5 mM isobutylmethylxanthine, 10 ng/ml N2 solution, 10 μM retinoic acid and 100 μM ascorbic acid.

Forty-eight hours following differentiation, the mesenchymal stem cells showed neuronal-like morphology, including long processes and dendrites (Fig. 3). Twenty-four hours prior to transplantation, cells were transfected with 5 mg/ml iron-oxide nanoparticles (Guerbet Laboratories, France) using Fugene reagent (1 microliter/ml) in DMEM medium. As a control, MSC and neuronal-differentiated MSC (0.2×10^6) were injected into naive mice ($n = 6$ in each mice group). Forty-five days after transplantation, mice were sacrificed. The mice were anesthetized and perfused intracardially with saline followed by 4% PFA in PBS. Brains were placed in 4% paraformaldehyde 0.1 M phosphate buffer, pH 7.4, 4 °C and stored in 30% sucrose in a 0.1 M phosphate buffer, pH 7.4, 4 °C for 3–4 days and then immersed in Methyl Butane and stored at −70 °C until they were cryosectioned. Histological studies demonstrated that most of the GFP-positive cells were located around the transplanted area in the striatum. However, transplanted cells were clearly seen in the contralateral striatum around the 6-OHDA lesion. Moreover, we found GFP cells along the path of migration from the left striatum, through the corpus callosum, ending in the right striatum, thalamic nuclei and substantia nigra (Fig. 4A–D).

The presence of the transplanted bone marrow derived stem cells was also analyzed by detecting the iron-transfected cells in situ; slides were placed in potassium ferrocanide 4% with equal volume of 1.2 mmol/L hydrochloride acid solution (Sigma MA-HT20) for 10 min, rinsed in deionized water and then stained with pararosaniline solution for 3–5 min. The iron staining, similar to the GFP labeling, showed iron-positive MSC in the injected striatum and a significant number of iron-positive MSC in the contralateral striatum around the 6-OHDA lesion and in the path of the migration (Fig. 4A–D). MSC and neuronal-differentiated MSC were seen to migrate similarly and to populate the 6-OHDA lesioned hemisphere. GFP transplanted cells were counted in the brains of mice transplanted with MSC and neuronal-differentiated cells using thirty histological slides (10 μm wide) for each brain taken sequentially from a brain slice starting 0.14 mm rostral to the bregma and ending −2.44 mm caudal to the bregma. For each brain the average was calculated (Fig. 4E). There was no significant difference in the migration of the mouse MSC as compared to the neuronal differentiated cells.

Thus, we hereby report, for the first time to our knowledge, that bone marrow derived stem cells, transplanted into the striatum of a mouse model of Parkinson's disease survive for a few months and migrate to the lesioned area in the contralateral hemisphere.

The capability of bone marrow stem cells to migrate has already been demonstrated in other animal models. Azizi et al. demonstrated migration of transplanted human marrow stem cells in a rat brain along known pathways for migration of neural stem cells to the successive layers of the brain [1]. The donor cells were found in multiple areas of the brain including the contralateral cortex but were mainly concentrated in the striatum and along the corpus callosum. Kopen et al. [14] injected mouse marrow mesenchymal cells into the lateral ventricle of neonatal mice, and after injection, the cells mimicked the behavior of neural progenitors, by migrating along established routes of intrinsic progenitor cells, to reach the striatum, cortex and cerebellum. However, injection of marrow mesenchymal cells into the lateral ventricle may have enabled cells to gain access to these various regions of the brain, through the cerebrospinal fluid, without having to migrate. Fallon et al. showed massive migration and differentiation of intrinsic neuroprogenitor cells to dopaminergic neurons in the substantia nigra of a 6-OHDA lesion model in rats, following infusion of transforming growth factor alpha into forebrain structures [5].

The mechanism of migration has been investigated. The central nervous system has traditionally been regarded as an

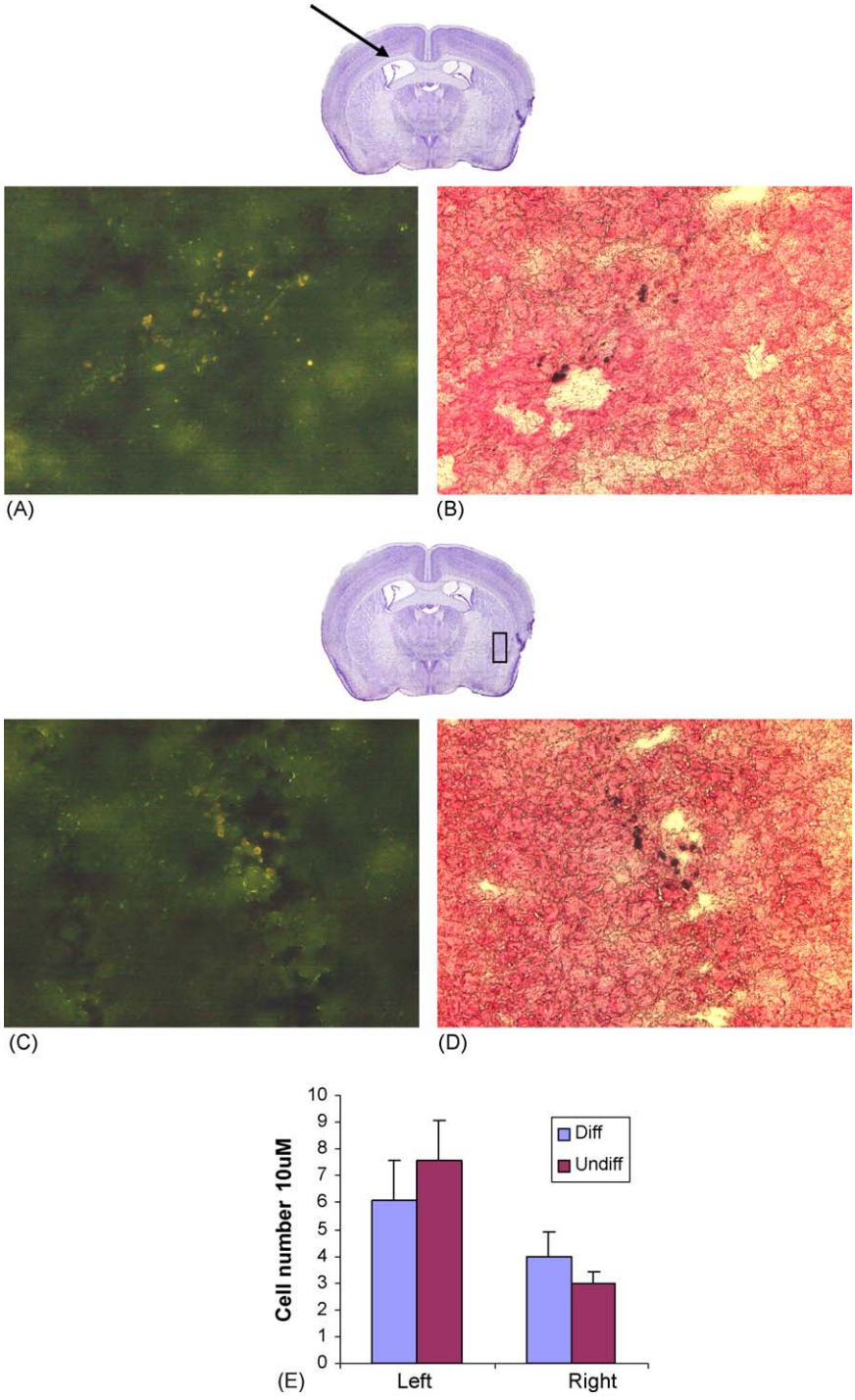

Fig. 4. Migration of transplanted bone marrow cells from the left striatum, through the corpus callosum to the 6-OHDA lesioned right striatum. (A) Enhanced GFP mouse bone marrow mesenchymal cells in the left corpus callosum. (B) Iron staining of differentiated mouse bone marrow mesenchymal cells in the left corpus callosum. (C) Enhanced GFP mouse bone marrow mesenchymal cells in the right striatum. (D) Iron staining of the differentiated mouse bone marrow mesenchymal cells in the right striatum. (E) Evaluation of the number of GFP-transplanted MSC and differentiated cells in the lesion side (right) and transplanted side (left) (average ± S.E.).

immunologically privileged organ because of a relatively impermeable blood-brain barrier and an immunosuppressive microenvironment, which limits immune cell entry and function [3]. 6-OHDA is known, however, to induce an inflammatory process with proliferation of reactive microglia in the substantia nigra pars compacta [7]. It is assumed that these reactive microglia induce the inflammatory process and release chemotactic factors, including the stromal cell-derived factor 1 (SDF-1). This alpha-chemokine stimulates migration of hematopoietic progenitor cells in the development of the immune system. Indeed, SDF-1 has been shown to be highly expressed in the nervous system, and several studies have revealed that SDF-1 plays a

crucial role in neocortical neural cell migration, such as during cerebellar embryogenesis [21]. Similarly, Peng et al. showed that SDF-1 alpha can induce human neuronal progenitor cell chemotaxis in vitro [17]. This suggests that the inflammation induced by tissue damage may provide a stimulus that recruits regenerative cells.

MSC have been shown to migrate to the ischemic hemisphere when injected intravenously with acute stroke lesions in rodents, and to reduce neurological deficit [4]. These cells appear to selectively enter the brain through the intact blood brain barrier, and alter the brain function. Hill et al. showed that SDF-1 and its receptor CXCR4 are implicated in the homing of bone marrow-derived cells to sites of injury in an animal stroke model [10]. They demonstrated localized SDF-1 expression in the ischemic penumbra, in mice that received bone marrow transplants from GFP transgenic donors, prior to a temporary middle cerebral artery suture occlusion. Since it has been shown that CXCR4 receptors are present on the MSC membrane, we suggest that the SDF-1 gradient from the 6-OHDA lesion might stimulate the migration of the bone marrow stem cell to the injured area.

The rationale behind cell delivery to promote tissue repair is based on the belief that endogenous repair is insufficient in several pathological conditions. Therefore, cell transplantation may contribute to tissue functional recovery in various neurodegenerative diseases. In the current study we have shown the homing capability of artificially administered bone marrow stem cells. These findings, along with the studies that showed the potential of MSC to differentiate into dopaminergic-like neurons, indicate a promising therapy for Parkinson's disease.

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